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Establishment and characterization of embryogenic cell  
suspension cultures from immature and mature embryos of barley  
(*Hordeum vulgare L.*).

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JOURNAL: Plant Cell Tissue and Organ Culture 32 (1):p19-25 1993

ISSN: 0167-6857

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RECORD TYPE: Abstract

LANGUAGE: English

**ABSTRACT:** Two finely-dispersed, homogeneous and regenerable cell suspension cultures were established from embryogenic callus derived from immature and mature embryos in barley. The quality and viability of suspension cells obtained were determined using differential-interference-contrast microscope and fluorescence microscope. Cell suspension cultures, maintained in modified liquid CC medium, showed a 10-fold increase in dry weight after two weeks with a doubling time of about 3 days. Addition of L-proline and casein hydrolysate in the medium had positive effect on the growth of cell cultures. Subculture interval significantly affected mitotic index. Both cell lines established were able to regenerate plants by somatic embryogenesis, but cell line Z-IM showed much higher regeneration capacity than cell line Z-M. Comparatively high frequencies of variations in chromosome number and structure were found in both lines, and a correlation between karyotype and morphogenic capacity was noticed.

1993

5/3, K, AB/11 (Item 11 from file: 155)  
DIALOG(R) File 155: MEDLINE(R)

03784689 83155573 PMID: 6831524

Cell type-specific binding of Ricinus lectin to murine cerebellar cell surfaces in vitro.

Sack HJ; Stohr M; Schachner M  
Cell and tissue research (GERMANY) 1983, 228 (1) p183-204, ISSN  
0302-766X Journal Code: CQD

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The binding of several plant lectins, Concanavalin A (Con A), Lens culinaris A (LCA), wheat germ agglutinin (WGA), and Ricinus communis agglutinin 120 (RCA 120) to cell surfaces of developing mouse cerebellar cells was assayed by the use of fluorescein isothiocyanate (FITC)-conjugated compounds. Freshly dissociated, live single-cell suspensions from 6-day-old mouse cerebellum contain 93% ConA, 99% LCA, 98% WGA, and 59% RCA 120-positive cells with ring fluorescence. Of the RCA 120-positive cells, 4% express a high and 55% a lower or very low number of lectin receptors. Flow cytometric analysis of fluorescent lectin binding yields results qualitatively similar to those obtained by scoring positive and negative cells in the fluorescence microscope. In monolayer cultures of 6-day-old mouse cerebellum practically all cells express receptors for ConA, LCA, and WGA, whereas RCA 120 binding sites are absent from neurons with small cell bodies (granule, basket and stellate cells) and present in large number on neurons with large cell bodies (Purkinje and possibly Golgi Type-II cells) and fibroblasts. RCA 120 receptors are weakly expressed on astro- and oligodendroglia. Cell type-specific expression of RCA 120 receptors is constant throughout all ages studied (embryonic day 13 to postnatal day 9). At early embryonic ages the proportion of highly fluorescent neurons with large cell bodies is significantly increased.

4415551 82031828 PMID: 7026684

Cell sorter immunofluorescence detection of human erythrocytes labelled in suspension with antibodies specific for hemoglobin S and C.  
Bigbee WL; Branscomb EW; Weintraub HB; Papayannopoulou T;  
Stamatoyannopoulos G

Journal of immunological methods (NETHERLANDS) 1981, 45 (2) p117-27,  
ISSN 0022-1759 Journal Code: IFE

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

We have developed an immunochemical method for labeling human red blood cells in suspension with hemoglobin-specific antibodies. A membrane permeable cross-linking reagent, dimethyl suberimidate, is used to covalently bind, *in situ*, a fraction of the intracellular hemoglobin to integral membrane proteins. Hypotonic lysis and washing of the cells removes the unbound hemoglobin resulting in red blood cell ghosts which are permeable to macromolecules. Fluorescein-labeled antibodies for the hemoglobin variants S and C bind specifically to hemoglobin AS and AC ghosts, respectively, and not to normal hemoglobin AA ghosts. This technique can be used to prepare ghost **suspensions** for cell sorter analysis in which large numbers (10(9)--10(10)) of normal ghosts can be rapidly screened for the presence of rare anti-hemoglobin S and anti-hemoglobin C binding ghosts. In reconstruction experiments using mixtures of AS and AA cells and anti-hemoglobin S, AS ghosts as rare as 3 X 10(-5) were quantitatively recovered. Fluorescence artifacts prevented direct enumeration of AS ghosts at lower frequencies, but a two-step flow sorting-fluorescence microscope visual scanning procedure allows semiquantitative detection of anti-hemoglobin S-labeled ghosts as low as 10(--7). This method can be used for rapidly screening blood samples from individuals of normal hemoglobin A genotype for the presence of rare anti-hemoglobin S and anti-hemoglobin C binding ghosts.

... and not to normal hemoglobin AA ghosts. This technique can be used to prepare ghost **suspensions** for cell sorter analysis in which large numbers (10(9)--10(10)) of normal ghosts can be...

Intracellular compartmentalization of fura-2 dye demonstrated by laser-excitation fluorescence microscopy: a problem in measuring cytosolic free calcium concentration using fura-2 fluorescence in vascular smooth muscle cells.

Takeuchi K; Sato SI; Abe K; Kimura M; Abe TA; Yoshinaga K; Inaba H  
Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan.

Tohoku journal of experimental medicine (JAPAN) Sep 1989, 159 (1)  
p23-35, ISSN 0040-8727 Journal Code: VTF

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Recently, we have developed a novel laser-excitation fluorescence microscope system to study intracellular calcium ( $Ca^{2+}$ ) in individual cultured vascular smooth muscle (VSM) cells using fluorescent indicators for ( $Ca^{2+}$ ). In the course of our study, it was shown that the subcellular fluorescence distribution of fura-2 was not homogeneous in VSM cells incubated with the acetoxy-methyl ester form of fura-2, fura-2/AM. The fluorescence appeared spotty or filamentous and resembled in shapes the intracellular organelles, suggesting that there was fura-2 dye compartmentalization in the organelles. To clarify the nature of the subcellular fluorescence, the soluble fraction of cells loaded with the dye was analyzed through high performance liquid chromatography (HPLC). We also examined the excitation spectra of fluorescence in the soluble fraction, which was compared with that in the cell suspension. Using HPLC, it has been shown that no other than fura-2 was found in the soluble fraction, whereas analyses of excitation spectra have indicated that the membrane fraction contained fura-2/AM or its lipophilic metabolite. On the other hand, indo-1 dye fluorescence showed a diffuse intracellular distribution, but the nuclear region had higher or sometimes lower fluorescence levels than the cytoplasm. The present results suggest that it may be necessary to assess subcellular fura-2 compartmentalization and possible interference by fura-2 AM or its lipophilic metabolite for the accurate measurement of intracellular  $Ca^{2+}$  concentration in VSM cells. It is also suggested that indo-1 may be more suitable for estimating  $Ca^{2+}$  concentration than fura-2 in individual VSM cells.

05894098 87110771 PMID: 3543135

Methanol fixation permits flow cytometric analysis of immunofluorescent stained intracellular antigens.

Levitt D; King M

Journal of immunological methods (NETHERLANDS) Feb 11 1987, 96 (2)

p233-7, ISSN 0022-1759 Journal Code: IFE

Contract/Grant No.: AM-35050, AM, NIADDK

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Fixation and immunofluorescent staining methods were developed for analyzing intracellular antigens with the cell flow cytometer. Fixing **cell suspensions** with 100% methanol provided best preservation of morphology, lowest fluorescent background staining and most intense specific immunofluorescence. Immunoglobulins present in B cell lines that were representative of different developmental stages could be distinguished quantitatively. **Fluorescence histograms** were compared with **fluorescence microscope** presentation of stained cells.

Intracellular antigens that reacted with monoclonal antibodies could also be evaluated by flow cytometry. This method was utilized to assess plasmacyte development in mouse spleen cell cultures after stimulation with lipopolysaccharide.

... immunofluorescent staining methods were developed for analyzing intracellular antigens with the cell flow cytometer. Fixing **cell suspensions** with 100% methanol provided best preservation of morphology, lowest fluorescent background staining and most intense...

5/3,K,AB/6 (Item 6 from file: 155)  
DIALOG(R) File 155: MEDLINE(R)

06344488 88059247 PMID: 3680375

Fura-2 measurement of cytosolic free  $\text{Ca}^{2+}$  in monolayers and suspensions of various types of animal cells.

Malgaroli A; Milani D; Meldolesi J; Pozzan T

Department of Pharmacology, University of Milan, Italy.

Journal of cell biology (UNITED STATES) Nov 1987, 105 (5) p2145-55,

ISSN 0021-9525 Journal Code: HMV

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The fluorescent indicator fura-2 has been applied to a variety of cell types in order to set up appropriate conditions for measurements of the cytosolic concentration of free ionized  $\text{Ca}^{2+}$  [ $[\text{Ca}^{2+}]_i$ ] in both cell suspensions and single cells analyzed in a conventional fluorimeter or in a fluorescence microscope equipped for quantitative analyses (with or without computerized image analyses), respectively. When the usual procedure for fluorescence dye loading (i.e., incubation at 37 degrees C with fura-2 acetoxy-methyl ester) was used, cells often exhibited a nonhomogeneous distribution of the dye, with marked concentration in multiple small spots located preferentially in the perinuclear area. These spots (studied in detail in human skin fibroblasts), were much more frequent in attached than in suspended cells, and were due to the accumulation (most probably by endocytosis) of the dye within acidic organelles after hydrolysis by lysosomal enzyme(s). When loading with fura-2 was performed at low (15 degrees C) temperature, no spots appeared, and cells remained diffusely labeled even after subsequent incubation at 32-37 degrees C for up to 2 h. Homogeneous distribution of the dye is a prerequisite for appropriate  $[\text{Ca}^{2+}]_i$  measurement. In fact, comparison of the results obtained in human skin fibroblasts labeled at either 37 or 15 degrees C demonstrated in spotty cells a marked apparent blunting of  $\text{Ca}^{2+}$  transients evoked by application of bradykinin. Additional problems were encountered when using fura-2. Leakage of the dye from loaded cells to the

ee HELP NEWS 340 for details.

| Set  | Items                      | Description                |
|--|----------------------------|----------------------------|
| ? s  | fluorescen? (5n)microspe   |                            |
| 565974   | FLUORESCEN?                |                            |
| 3  | MICROSPE                   |                            |
| S1   | 0                          | FLUORESCEN? (5N)MICROSPE   |
| ? s  | fluorescen? (5n)microscope |                            |
| 565974   | FLUORESCEN?                |                            |
| 111005   | MICROSCOPE                 |                            |
| S2   | 4268                       | FLUORESCEN? (5N)MICROSCOPE |
| ? s  | cell(2n)suspension??       |                            |
| 4297647  | CELL                       |                            |
| 197125   | SUSPENSION??               |                            |
| S3   | 31604                      | CELL (2N) SUSPENSION??     |
| ? s  | s2 and s3                  |                            |
| 4268   | S2                         |                            |
| 31604  | S3                         |                            |
| S4   | 27                         | S2 AND S3                  |
| ? rd   |                            |                            |
| >>>Duplicate detection is not supported for File 340.                                  |                            |                            |
| >>>Records from unsupported files will be retained in the RD set.                      |                            |                            |
| ...completed examining records   |                            |                            |
| S5   | 18                         | RD (unique items)          |
| ? t  | s5/3,k,ab/1-18             |                            |
| 5/3,K,AB/1   | (Item 1 from file: 155)    |                            |
| DIALOG(R)File 155: MEDLINE(R)  |                            |                            |
| 12778000   | 21669431                   | PMID: 11809120             |
| [Antigen presentation of eosinophils in mice]  |                            |                            |
| Shi H; Deng J; Qin S; et Al  |                            |                            |
| Respiratory Department, First Affiliated Hospital, Guangxi Medical                     |                            |                            |
| University, Nanning 530021, China.   |                            |                            |
| Zhonghua yi xue za zhi (China) May 2001, 81 (9) p544-8, ISSN                           |                            |                            |
| 0376-2491  | Journal Code: 7511141      |                            |
| Languages: CHINESE   |                            |                            |
| Document type: Journal Article   |                            |                            |
| Record type: In Process  |                            |                            |
| OBJECTIVE: To explore whether or not eosinophils can present antigen to T              |                            |                            |
| lymphocytes in vivo, and to further elucidate the process and                          |                            |                            |
| characteristics of antigen-presentation by eosinophils in vivo. METHODS:               |                            |                            |
| BALB/c mice were sensitized and challenged by ovalbumin to recruit                     |                            |                            |
| eosinophil infiltration into the airways. The airway eosinophils were                  |                            |                            |
| purified and were labeled with a fluorescent dye. The labeled eosinophils              |                            |                            |
| were instilled into the mouse tracheas, <b>fluorescent microscope</b>                  |                            |                            |
| was used to observe the migration of endobronchial eosinophils in vivo.                |                            |                            |
| Single cell suspension was prepared from paratracheal lymph                            |                            |                            |
| nodes of mice receiving antigen-exposed eosinophil instillation, and flow              |                            |                            |
| cytometry was used to determine proliferation response of T cells and to               |                            |                            |
| identify the subset of responding T cells. RESULTS: By 8 h after tracheal              |                            |                            |
| instillation, labeled eosinophils were visible in the subcapsular region               |                            |                            |
| and streaming through the subcapsular sinus (19.0 +/- 1.8/mm <sup>2</sup> ). With      |                            |                            |
| increasing time, the numbers of eosinophils entering the regional lymph                |                            |                            |
| nodes increased, peaking at 24 h (59.2 +/- 7.2/mm <sup>2</sup> ) and persisting for at |                            |                            |
| least 120 h (29.6 +/- 2.8/mm <sup>2</sup> ). In sensitized mice that received 5 x      |                            |                            |
| 10(5) antigen-exposed eosinophils, in vivo percentage of proliferating T               |                            |                            |
| cell in the paratracheal lymph nodes 1 d after eosinophil instillation                 |                            |                            |
| (6.9% +/- 0.5%) were much higher than basic control value (3.2% +/- 0.3%, P            |                            |                            |
| < 0.01), peaked at day 3 (10.8% +/- 0.8%, P < 0.01), and then declined over            |                            |                            |
| 7 d (6.1% +/- 0.6%, P < 0.05). Eosinophil-induced in vivo T cell                       |                            |                            |
| proliferation was antigen-specific, and the responding T cells were limited            |                            |                            |

to CD4(+) cells. CONCLUSIONS: Eosinophils within the lumina of airways can process inhaled antigens, traffick to regional lymph nodes and function in vivo as antigen-presenting cells to stimulate responses of CD4(+) T cells.

... were labeled with a fluorescent dye. The labeled eosinophils were instilled into the mouse tracheas, fluorescent microscope was used to observe the migration of endobronchial eosinophils in vivo. Single cell suspension was prepared from paratracheal lymph nodes of mice receiving antigen-exposed eosinophil instillation, and flow...

5/3,K,AB/2 (Item 2 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)

11629742 21217761 PMID: 11321461  
In vivo identification of parasinus macrophages in the mesenteric lymph